

In the Claims:

1. A process for detecting threonine or serine kinase activity in an immunoassay

comprising the following steps:

a) providing a protein or peptide comprising the sequence motif

-Z-X-Y- or -Y-X-Z-

wherein

Z = threonine or serine

X = a sequence of amino acids, preferably between 1 and 1000 amino acids, which may be the same or different

Y = tyrosine, threonine or serine

as a substrate for threonine or serine kinase, said protein or peptide being pre-phosphorylated at the Y position;

b) incubating the protein or peptide with a phosphate donor and a threonine or serine kinase to form a protein or peptide which is phosphorylated at positions Y and Z;

c) adding an antibody having a specificity to a peptide or protein which is phosphorylated at the Y and Z position; and

d) detecting the threonine or serine kinase activity.

2. The process according to claim 1, wherein the phosphate donor is ATP, GTP, or a synthetic cosubstrate.

3. The process according to claim 1, wherein the immunoassay is performed as a direct binding immunoassay, preferably a homogeneous direct binding immunoassay.

4. The process according to claim 3, wherein a labelled peptide or protein is used as a substrate.

5. The process according to claim 3, wherein a labelled antibody is used.

6. The process according to claim 4, wherein the peptide/protein or antibody is labelled by a luminescent tag, a radioactive marker, a reporter enzyme or an affinity ligand.


7. The process according to claim 1, wherein the immunoassay is performed as an indirect binding immunoassay, preferably a homogeneous indirect binding immunoassay.


8. The process according to claim 7, wherein a labelled ligand which is phosphorylated at its Y and Z position (bis-phosphorylated ligand) is added to compete with the protein or peptide which is phosphorylated at its Y and Z position (bis-phosphorylated protein or peptide) for binding to the antibody.

9. The process according to claim 8, wherein the bis-phosphorylated ligand is labelled by a luminescent tag, a radioactive marker, a reporter enzyme or an affinity ligand.

10. The process according to claim 9 wherein the ligand comprises Lys-Phe-Met-Met-pThr-Pro-pTyr-Val-Val-Thr-Arg-NH₂, and particular is 5-Tamra-Ahx-Lys-Phe-Met-Met-pThr-Pro-pTyr-Val-Val-Thr-Arg-NH₂.

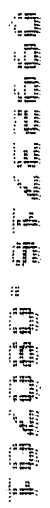
11. The process according to claim 1, wherein the assay is performed as a fluorescence immunoassay, in particular a fluorescence polarization immunoassay, a fluorescence correlation spectroscopic assay, a fluorescence resonance energy transfer assay, or a fluorescence intensity distribution assay.


12. The process according to claim 1, wherein X in the sequence motif comprises proline or glutamate or glycine.


13. The process according to claim 1, wherein the protein provided is JNK1, JNK2 or JNK3 protein.

14. The process according to claim 1, wherein the peptide provided includes sequences identical to those of the JNK1, JNK2 or JNK3 active-site loop.

15. The process according to claim 1, wherein the peptide comprises the amino acid sequence H-Lys-Phe-Met-Met-Thr-Pro-pTyr-Val-Val-Thr-Arg-NH₂ (p means phosphorylated).


~~16. The process according to claim 1, wherein the peptide is composed of the amino acid sequence H-Lys-Phe-Met-Met-Thr-Pro-pTyr-Val-Val-Thr-Arg-NH₂ (p means phosphorylated).~~

17. The process according to claim 1, wherein the incubation of the protein or peptide is carried out in the presence of a threonine kinase.

18. The process according to claim 17, wherein the threonine kinase is a mitogen-activated protein kinase kinase (MKK)(also named stress activated protein kinase kinase, SKK).

19. The process according to claim 18, wherein the kinase is MKK7 (also named SKK4).

20. The process according to claim 1, wherein the antibody is a monoclonal or polyclonal antibody.

21. The process according to claim 20, wherein the antibody is a polyclonal antibody.

22. The process according to claim 21, wherein the antibody is a polyclonal antibody specific for bis-phosphorylated, in particular active JNK.

23. The process according to claims 1, wherein steps a) to d) are performed sequentially.

24. A kit for detecting threonine or serine kinase activity in an immunoassay comprising the following components:

- a substrate as defined in claim 1;
- an antibody as defined in claim 1.

25. The kit according to claim 24 further comprising a threonine or serine kinase, and/or reaction buffers including a phosphate donor, preferably ATP.

26. The kit according to claim 24, further comprising a labelled ligand, preferably luminescently labelled ligand, said ligand comprising the following sequence motif

-Z-X-Y- or -Y-X-Z-

wherein

Z = threonine or serine

X = a sequence of amino acids, preferably between 1 and 1000 amino acids, which may be the same or different

Y = tyrosine, threonine or serine

said protein or peptide ligand being phosphorylated at the Z and Y positions;

